

WHAT IS CLAIMED IS:

1           1. An isolated infectious chimeric respiratory syncytial virus (RSV)  
2 comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large  
3 polymerase protein (L), a RNA polymerase elongation factor, and a partial or complete  
4 RSV background genome or antigenome of a human or bovine RSV combined with one  
5 or more heterologous gene(s) and/or genome segment(s) of a different RSV to form a  
6 human-bovine chimeric RSV genome or antigenome.

1           2. The chimeric RSV of claim 1, wherein said one or more  
2 heterologous gene(s) and/or genome segment(s) include one or more RSV NS1, NS2, N,  
3 P, M, SH, M2(ORF1), M2(ORF2), L, F or G gene(s) or genome segment(s) or a leader,  
4 trailer or intergenic region of the RSV genome or a segment thereof.

1           3. The chimeric RSV of claim 2, wherein said one or more  
2 heterologous gene(s) and/or genome segment(s) includes one or more gene(s) or genome  
3 segment(s) encoding a RSV F, G and/or SH glycoprotein or an immunogenic domain or  
4 epitope thereof.

1           4. The chimeric RSV of claim 1, wherein the human-bovine chimeric  
2 RSV genome or antigenome encodes a chimeric glycoprotein having both human and  
3 bovine glycoprotein domains or immunogenic epitopes.

1           5. The chimeric RSV of claim 4, wherein said one or more  
2 heterologous gene(s) and/or genome segment(s) includes a gene segment encoding a  
3 glycoprotein ectodomain.

1           6. The chimeric RSV of claim 1, wherein a heterologous gene or  
2 genome segment is substituted for a counterpart gene or genome segment in a partial RSV  
3 background genome or antigenome.

1           7. The chimeric RSV of claim 1, wherein a heterologous gene or  
2 genome segment is added adjacent to, within, or as a replacement to, a noncoding region  
3 of the partial or complete RSV background genome or antigenome.

1           8.       The chimeric RSV of claim 1, wherein a heterologous gene or  
2    genome segment is added or substituted at a position corresponding to a wild-type gene  
3    order position of a counterpart gene or genome segment within the partial or complete  
4    RSV background genome or antigenome.

1           9.       The chimeric RSV of claim 1, wherein a heterologous gene or  
2    genome segment is added or substituted at a position that is more promoter-proximal or  
3    promoter-distal compared to a wild-type gene order position of a counterpart gene or  
4    genome segment within the partial or complete RSV background genome or antigenome.

1           10.      The chimeric RSV of claim 1, wherein the chimeric genome or  
2    antigenome comprises a partial or complete human RSV background genome or  
3    antigenome combined with one or more heterologous gene(s) and/or genome segment(s)  
4    from a bovine RSV.

1           11.      The chimeric RSV of claim 10, wherein one or more genes selected  
2    from N, P, NS1, NS2, M2-1 and M of a human RSV is/are replaced by one or more  
3    heterologous gene(s) from a bovine RSV.

1           12.      The chimeric RSV of claim 11, wherein both N and P genes of a  
2    human RSV are replaced by counterpart N and P genes from a bovine RSV.

1           13.      The chimeric RSV of claim 11, wherein both NS1 and NS2 genes  
2    of a human RSV are replaced by counterpart NS1 and NS2 genes from a bovine RSV.

1           14.      The chimeric RSV of claim 11, wherein two or more of the M2-1,  
2    M2-2 and L genes are replaced by counterpart genes from a bovine RSV

1           15.      The chimeric RSV of claim 11, wherein each of the N, P, NS1,  
2    NS2, M2-1 and M genes of a human RSV are replaced by counterpart N, P, NS1, NS2,  
3    M2-1 and M genes from a bovine RSV.

1           16.      The chimeric RSV of claim 1, wherein the chimeric genome or  
2    antigenome comprises a partial or complete bovine RSV background genome or  
3    antigenome combined with one or more heterologous gene(s) and/or genome segment(s)  
4    from a human RSV.

1           17. The chimeric RSV of claim 16, wherein one or more human RSV  
2   glycoprotein genes selected from F, G and SH, or one or more genome segment(s)  
3   encoding cytoplasmic domain, transmembrane domain, ectodomain or immunogenic  
4   epitope portion(s) of F, G, and/or SH is/are added or substituted within a partial or  
5   complete bovine RSV background genome or antigenome.

1           18. The chimeric RSV of claim 17, wherein one or both human RSV  
2   glycoprotein genes F and G is/are substituted to replace one or both counterpart F and G  
3   glycoprotein genes in a partial bovine RSV background genome or antigenome.

1           19. The chimeric RSV of claim 17, wherein the human-bovine  
2   chimeric genome or antigenome incorporates antigenic determinants from one or both  
3   subgroup A and subgroup B human RSV.

1           20. The chimeric RSV of claim 17, wherein both human RSV  
2   glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein  
3   genes in the bovine RSV background genome or antigenome.

1           21. The chimeric RSV of claim 20, which is rRSV/A2.

1           22. The chimeric RSV of claim 9, wherein one or more human RSV  
2   glycoprotein genes selected from F, G and SH is/are added or substituted at a position that  
3   is more promoter-proximal compared to a wild-type gene order position of a counterpart  
4   gene or genome segment within a partial or complete bovine RSV background genome or  
5   antigenome.

1           23. The chimeric RSV of claim 22, wherein both human RSV  
2   glycoprotein genes G and F are substituted at gene order positions 1 and 2, respectively,  
3   to replace counterpart G and F glycoprotein genes deleted at wild type positions 7 and 8,  
4   respectively in a partial bovine RSV background genome or antigenome.

1           24. The chimeric RSV of claim 23, which is rRSV/A2-G1F2

1           25. The chimeric RSV of claim 17, wherein the chimeric genome or  
2   antigenome is further modified by addition or substitution of one or more additional  
3   heterologous gene(s) or genome segment(s) from a human RSV within the partial or

4 complete bovine background genome or antigenome to increase genetic stability or alter  
5 attenuation, reactogenicity or growth in culture of the chimeric virus.

1 26. The chimeric RSV of claim 16, wherein one or more human RSV  
2 envelope-associated genes selected from F, G, SH, and M is/are added or substituted  
3 within a partial or complete bovine RSV background genome or antigenome.

1 27. The chimeric RSV of claim 26, wherein one or more human RSV  
2 envelope-associated genes selected from F, G, SH, and M is/are added or substituted  
3 within a partial bovine RSV background genome or antigenome in which one or more  
4 envelope-associated genes selected from F, G, SH, and M is/are deleted.

1 28. The chimeric RSV of claim 27, wherein human RSV envelope-  
2 associated genes F, G, and M are added within a partial bovine RSV background genome  
3 or antigenome in which envelope-associated genes F, G, SH, and M are deleted.

1 29. The chimeric RSV of claim 28, which is rRSV/A2-MGF.

1 30. The chimeric RSV of claim 1, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 present within a panel of mutant human RSV strains, said panel comprising cpts RSV 248  
4 (ATCC VR 2450), cpts RSV 248/404 (ATCC VR 2454), cpts RSV 248/955 (ATCC VR  
5 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV 530/1009 (ATCC VR 2451), cpts RSV  
6 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5 (ATCC VR 2542), and RSV B-1 cp-23  
7 (ATCC VR 2579).

1 31. The chimeric RSV of claim 30, wherein the chimeric genome or  
2 antigenome incorporates attenuating mutations adopted from different mutant RSV  
3 strains.

1 32. The chimeric RSV of claim 1, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 specifying an amino acid substitution at Val267 in the RSV N gene, Glu218 and/or  
4 Thr523 in the RSV F gene, Asn43, Cys319, Phe 521, Gln831, Met1169, Tyr1321 and/or  
5 His 1690 in the RSV polymerase gene L, and a nucleotide substitution in the gene-start  
6 sequence of gene M2.

1           33. The chimeric RSV of claim 32, wherein the chimeric genome or  
2 antigenome incorporates at least two attenuating mutations.

1           34. The chimeric RSV of claim 32, wherein the chimeric genome or  
2 antigenome includes at least one attenuating mutation stabilized by multiple nucleotide  
3 changes in a codon specifying the mutation.

1           35. The chimeric RSV of claim 1, wherein the chimeric genome or  
2 antigenome further comprises a nucleotide modification specifying a phenotypic change  
3 selected from a change in growth characteristics, attenuation, temperature-sensitivity,  
4 cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

1           36. The chimeric RSV of claim 35, wherein the nucleotide  
2 modification alters a SH, NS1, NS2, M2ORF2, or G gene of the chimeric virus.

1           37. The chimeric RSV of claim 36, wherein a SH, NS1, NS2, M2  
2 ORF2, or G gene of the chimeric virus is deleted in whole or in part or expression of the  
3 gene is ablated by introduction of one or more stop codons in an open reading frame of  
4 the gene.

1           38. The chimeric RSV of claim 35, wherein the nucleotide  
2 modification comprises a nucleotide deletion, insertion, substitution, addition or  
3 rearrangement of a cis-acting regulatory sequence of a selected gene within the chimeric  
4 RSV genome or antigenome.

1           39. The chimeric RSV of claim 38, wherein a gene end (GE) signal of  
2 the NS1 or NS2 gene is modified.

1           40. The chimeric RSV of claim 35, wherein the nucleotide  
2 modification comprises an insertion, deletion, substitution, or rearrangement of a  
3 translational start site within the chimeric genome or antigenome.

1           41. The chimeric RSV of claim 40, wherein the translational start site  
2 for a secreted form of the RSV G glycoprotein is ablated.

1           42. The chimeric RSV of claim 35, wherein the chimeric genome or  
2 antigenome is modified to encode a non-RSV molecule selected from a cytokine, a T-

3 helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of  
4 eliciting a protective immune response in a mammalian host.

1 43. The chimeric RSV of claim 35, which incorporates one or more  
2 gene(s) and/or genome segment(s) from parainfluenza virus (PIV).

1 44. The chimeric RSV of claim 43, wherein the chimeric genome or  
2 antigenome encodes a PIV HN or F glycoprotein or immunogenic domain or epitope  
3 thereof.

1 45. The chimeric RSV of claim 44, wherein the chimeric genome or  
2 antigenome encodes an ectodomain or immunogenic epitope of HN or F of PIV1, PIV2,  
3 or PIV3.

1 46. The chimeric RSV of claim 1 which is a virus.

1 47. The chimeric RSV of claim 1 which is a subviral particle.

1 48. A method for stimulating the immune system of an individual to  
2 induce protection against RSV which comprises administering to the individual an  
3 immunologically sufficient amount of the chimeric RSV of claim 1 combined with a  
4 physiologically acceptable carrier.

1 49. The method of claim 48, wherein the chimeric RSV is administered  
2 in a dose of  $10^3$  to  $10^6$  PFU.

1 50. The method of claim 48, wherein the chimeric RSV is administered  
2 to the upper respiratory tract.

1 51. The method of claim 48, wherein the chimeric RSV is administered  
2 by spray, droplet or aerosol.

1 52. The method of claim 48, wherein the chimeric RSV is administered  
2 to an individual seronegative for antibodies to RSV or possessing transplacentally  
3 acquired maternal antibodies to RSV.

1 53. The method of claim 48, wherein the chimeric RSV elicits an  
2 immune response against either human RSV A or RSV B.

1                   54. The method of claim 48, wherein the chimeric RSV elicits an  
2 immune response against both human RSV A and RSV B.

1                   55. The method of claim 48, wherein the chimeric RSV elicits an  
2 immune response against either human RSV A or RSV B and is co-administered with an  
3 immunologically sufficient amount of a second attenuated RSV capable of eliciting an  
4 immune response against human RSV A or RSV B, whereby an immune response is  
5 elicited against both human RSV A and RSV B.

1                   56. The method of claim 55, wherein the chimeric RSV and second  
2 attenuated RSV are administered simultaneously as a mixture.

1                   57. An immunogenic composition to elicit an immune response against  
2 RSV comprising an immunologically sufficient amount of the chimeric RSV of claim 1 in  
3 a physiologically acceptable carrier.

1                   58. The immunogenic composition of claim 57, formulated in a dose of  
2  $10^3$  to  $10^6$  PFU.

1                   59. The immunogenic composition of claim 57, formulated for  
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1                   60. The immunogenic composition of claim 57, wherein the chimeric  
2 RSV elicits an immune response against either human RSV A or RSV B.

1                   61. The immunogenic composition of claim 57, wherein the chimeric  
2 RSV elicits an immune response against both human RSV A and RSV B

1                   62. The immunogenic composition of claim 57, wherein the chimeric  
2 RSV elicits an immune response against either human RSV A or RSV B and wherein the  
3 composition further comprises an immunologically sufficient amount of a second  
4 attenuated RSV capable of eliciting an immune response against human RSV A or RSV  
5 B, whereby the composition elicits an immune response against both human RSV A and  
6 RSV B.

1                   63. An isolated polynucleotide molecule comprising a chimeric RSV  
2 genome or antigenome which includes a partial or complete RSV background genome or

3 antigenome of a human or bovine RSV combined with one or more heterologous gene(s)  
4 or genome segment(s) of a different RSV to form a human-bovine chimeric RSV genome  
5 or antigenome.

1 64. The isolated polynucleotide of claim 63, wherein said one or more  
2 heterologous gene(s) and/or genome segment(s) include one or more RSV NS1, NS2, N,  
3 P, M, SH, M2(ORF1), M2(ORF2), L, F or G gene(s) or genome segment(s) or a leader,  
4 trailer or intergenic region of the RSV genome or a segment thereof.

1 65. The isolated polynucleotide of claim 63, wherein a heterologous  
2 gene or genome segment is substituted for a counterpart gene or genome segment in a  
3 partial RSV background genome or antigenome.

1 66. The isolated polynucleotide of claim 63, wherein a heterologous  
2 gene or genome segment is added adjacent to, within, or as a replacement to, a noncoding  
3 region of the partial or complete RSV background genome or antigenome.

1 67. The isolated polynucleotide of claim 63, wherein a heterologous  
2 gene or genome segment is added or substituted at a position that is more promoter-  
3 proximal or promoter-distal compared to a wild-type gene order position of a counterpart  
4 gene or genome segment within the partial or complete RSV background genome or  
5 antigenome.

1 68. The isolated polynucleotide of claim 63, wherein the chimeric  
2 genome or antigenome comprises a partial or complete human RSV background genome  
3 or antigenome combined with one or more heterologous gene(s) and/or genome  
4 segment(s) from a bovine RSV.

1 69. The isolated polynucleotide of claim 68, wherein one or more  
2 genes selected from N, P, NS1, NS2, M2-1 and M of a human RSV is/are replaced by one  
3 or more heterologous gene(s) from a bovine RSV.

1 70. The isolated polynucleotide of claim 68, wherein both N and P  
2 genes of a human RSV are replaced by counterpart N and P genes from a bovine RSV.

1                   71. The isolated polynucleotide of claim 68, wherein both NS1 and  
2 NS2 genes of a human RSV are replaced by counterpart NS1 and NS2 genes from a  
3 bovine RSV.

1                   72. The isolated polynucleotide of claim 68, wherein two or more of  
2 the M2-1, M2-2 and L genes are replaced by counterpart genes from a bovine RSV

1                   73. The isolated polynucleotide of claim 63, wherein the chimeric  
2 genome or antigenome comprises a partial or complete bovine RSV background genome  
3 or antigenome combined with one or more heterologous gene(s) and/or genome  
4 segment(s) from a human RSV.

1                   74. The isolated polynucleotide of claim 73, wherein one or more  
2 human RSV glycoprotein genes selected from F, G and SH, or one or more genome  
3 segment(s) encoding cytoplasmic domain, transmembrane domain, ectodomain or  
4 immunogenic epitope portion(s) of F, G, and/or SH is/are added or substituted within a  
5 partial or complete bovine RSV background genome or antigenome.

1                   75. The isolated polynucleotide of claim 74, wherein one or both  
2 human RSV glycoprotein genes F and G is/are substituted to replace one or both  
3 counterpart F and G glycoprotein genes in a partial bovine RSV background genome or  
4 antigenome.

1                   76. The isolated polynucleotide of claim 75, wherein both human RSV  
2 glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein  
3 genes in the bovine RSV background genome or antigenome.

1                   77. The isolated polynucleotide of claim 67, wherein one or more  
2 human RSV glycoprotein genes selected from F, G and SH is/are added or substituted at a  
3 position that is more promoter-proximal compared to a wild-type gene order position of a  
4 counterpart gene or genome segment within a partial or complete bovine RSV  
5 background genome or antigenome.

1                   78. The isolated polynucleotide of claim 77, wherein both human RSV  
2 glycoprotein genes G and F are substituted at gene order positions 1 and 2, respectively,

3 to replace counterpart G and F glycoprotein genes deleted at wild type positions 7 and 8,  
4 respectively in a partial bovine RSV background genome or antigenome.

1 79. The isolated polynucleotide of claim 73, wherein the chimeric  
2 genome or antigenome is further modified by addition or substitution of one or more  
3 additional heterologous gene(s) or genome segment(s) from a human RSV within the  
4 partial or complete bovine background genome or antigenome to increase genetic stability  
5 or alter attenuation, reactogenicity or growth in culture of the chimeric virus.

1 80. The isolated polynucleotide of claim 73, wherein one or more  
2 human RSV envelope-associated genes selected from F, G, SH, and M is/are added or  
3 substituted within a partial or complete bovine RSV background genome or antigenome.

1 81. The isolated polynucleotide of claim 80, wherein human RSV  
2 envelope-associated genes F, G, and M are added within a partial bovine RSV  
3 background genome or antigenome in which envelope-associated genes F, G, SH, and M  
4 are deleted.

1 82. The isolated polynucleotide molecule of claim 63, wherein the  
2 human-bovine chimeric genome or antigenome incorporates antigenic determinants from  
3 both subgroup A and subgroup B human RSV.

1 83. The isolated polynucleotide molecule of claim 63, wherein the  
2 chimeric genome or antigenome is further modified by incorporation of one or more  
3 attenuating mutations.

1 84. The isolated polynucleotide molecule of claim 63, further  
2 comprising a nucleotide modification specifying a phenotypic change selected from a  
3 change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation,  
4 plaque size, host-range restriction, or a change in immunogenicity.

1 85. The isolated polynucleotide molecule of claim 63, wherein a SH,  
2 NS1, NS2, M2ORF2, or G gene is modified.

1 86. The isolated polynucleotide molecule of claim 85, wherein the SH,  
2 NS1, NS2, M2 ORF2, or G gene is deleted in whole or in part or expression of the gene is  
3 ablated by introduction of one or more stop codons in an open reading frame of the gene.

1           87. The isolated polynucleotide molecule of claim 59, wherein the  
2 nucleotide modification comprises a nucleotide deletion, insertion, addition or  
3 rearrangement of a *cis*-acting regulatory sequence of a selected RSV gene within the  
4 chimeric RSV genome or antigenome.

1           88. A method for producing an infectious attenuated chimeric RSV  
2 particle from one or more isolated polynucleotide molecules encoding said RSV,  
3 comprising:

4           expressing in a cell or cell-free lysate an expression vector comprising an  
5 isolated polynucleotide comprising a partial or complete RSV background genome or  
6 antigenome of a human or bovine RSV combined with one or more heterologous gene(s)  
7 or genome segment(s) of a different RSV to form a human-bovine chimeric RSV genome  
8 or antigenome, and RSV N, P, L and RNA polymerase elongation factor proteins.

1           89. The method of claim 88, wherein the chimeric RSV genome or  
2 antigenome and the N, P, L and RNA polymerase elongation factor proteins are expressed  
3 by two or more different expression vectors.

1           90. The chimeric RSV of claim 1, wherein the bovine-human chimeric  
2 genome or antigenome comprises a partial or complete RSV vector genome or  
3 antigenome combined with one or more heterologous genes or genome segments  
4 encoding one or more antigenic determinants of one or more heterologous pathogens.

1           91. The chimeric RSV of claim 90, wherein said one or more  
2 heterologous pathogens is a heterologous RSV and said heterologous gene(s) or genome  
3 segment(s) encode(s) one or more RSV NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2),  
4 L, F or G protein(s) or fragment(s) thereof.

1           92. The chimeric RSV of claim 90, wherein the vector genome or  
2 antigenome is a partial or complete RSV A genome or antigenome and the heterologous  
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of a RSV B  
4 subgroup virus.

1           93. The chimeric RSV of claim 90, wherein the chimeric genome or  
2 antigenome incorporates one or more gene(s) or genome segment(s) of a BRSV that  
3 specifies attenuation.

1           94. The chimeric RSV of claim 90, wherein one or more HPIV1,  
2 HPIV2, or HPIV3 gene(s) or genome segment(s) encoding one or more HN and/or F  
3 glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s) thereof is/are added to  
4 or incorporated within the partial or complete HRSV vector genome or antigenome.

1           95. The chimeric RSV of claim 90, wherein a transcription unit  
2 comprising an open reading frame (ORF) of an HPIV2 HN or F gene is added to or  
3 incorporated within the chimeric HRSV vector genome or antigenome.

1           96. The chimeric RSV of claim 35, wherein the vector genome or  
2 antigenome is a partial or complete BRSV genome or antigenome and the heterologous  
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more  
4 HRSV(s).

1           97. The chimeric RSV of claim 96, wherein the partial or complete  
2 BRSV genome or antigenome incorporates one or more gene(s) or genome segment(s)  
3 encoding one or more HRSV glycoprotein genes selected from F, G and SH, or one or  
4 more genome segment(s) encoding cytoplasmic domain, transmembrane domain,  
5 ectodomain or immunogenic epitope portion(s) of F, G, and/or SH of HRSV.

1           98. The chimeric RSV of claim 90, wherein the vector genome or  
2 antigenome is a partial or complete HRSV or BRSV genome or antigenome and the  
3 heterologous pathogen is selected from measles virus, subgroup A and subgroup B  
4 respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2  
5 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus,  
6 Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza  
7 viruses.

1           99. The chimeric RSV of claim 98, wherein said one or more  
2 heterologous antigenic determinant(s) is/are selected from measles virus HA and F  
3 proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins,

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4 mumps virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2  
5 human immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus  
6 gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein  
7 Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and  
8 NS1 proteins, and alphavirus E protein, and antigenic domains, fragments and epitopes  
9 thereof.

1 100. The chimeric RSV of claim 99, wherein the heterologous pathogen  
2 is measles virus and the heterologous antigenic ~~determinant(s)~~ is/are selected from the  
3 measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1 101. The chimeric RSV of claim 100, wherein a transcription unit  
2 comprising an open reading frame (ORF) of a measles virus HA gene is added to or  
3 incorporated within a HRSV vector genome or antigenome.